

Preserving reproductive capacity in young boys: what is acceptable and practically possible

Dr. Hoong Wei Gan BM BS BMedSci (Hons) MRCPCH, Specialty Registrar in Paediatrics & Honorary Research Fellow in Paediatric Neuroendocrine Late Effects of Childhood Cancer

Dr. Helen A. Spoudeas MBBS DRCOG FRCP FRCPCH MD, Consultant in Neuroendocrine Late Effects of Childhood Cancer & Honorary Senior Lecturer in Paediatric Endocrinology

University College London Hospitals NHS Foundation Trust

We can now cure over 70% of all childhood cancers, and this cure rate approaches 90% for some tumours¹. Enhanced supportive care for dose-intense treatment regimens imposed on a cancer incidence stable over the last 40 years² are responsible for this success. Where survival is so high, however, further attempts to increase it must be balanced against the multi-organ toxicities to the majority of developing children. Though not necessarily impotent, 15-30% of male survivors are rendered infertile³, either from hypothalamopituitary-gonadal exposure to chemoradiation⁴ or from diseases such as Hodgkin's lymphoma⁵.

In today's society, 1 in 6 healthy couples seek help from reproductive clinics to conceive⁶ with rapid advancements in assisted reproductive technology (ART) being made in the last 2 decades. This includes cryopreservation of gametes and embryos prior to gonadotoxic cancer therapies to preserve fertility in adults. This article explores the possibilities and particular developmental and ethical issues surrounding sperm cryopreservation in young boys with cancer, and examines the unique legal implications of fertility counselling in adolescence.

The impact of childhood cancer treatment on male fertility

Spermatogenesis begins only at puberty. This process requires meiotic division of diploid spermatogonia to produce haploid spermatozoa, a process which continues thereafter throughout adult life. Spermatogenesis requires sufficient intratesticular testosterone production maintained by pituitary-derived follicular-stimulating hormone (FSH) and luteinising hormone (LH) with negative gonadal feedback from inhibin B (Sertoli cells) and testosterone (Leydig cells). Both disease- and treatment-related factors can damage the hypothalamopituitary-testicular axis at one or multiple levels (Figure 1), thereby compromising male reproductive capacity.

Chemotherapy

The rapidly dividing sperm-producing testicular seminiferous epithelium is highly susceptible to cytotoxic damage, its extent determined by drug type, cumulative drug dosage and patient age at exposure. By contrast, the testosterone-producing Leydig cells are relatively robust. Consequently, pubertal sexual development may proceed normally (and sometimes early), the only sign of gonadotoxicity and future subfertility being small testicular volumes relative to the degree of virilisation and pubertal stage.

Because cancer treatment protocols are multidrug regimens, the individual effect of specific drug types has proved difficult to accurately determine. However, most alkylating agents (e.g. cyclophosphamide, busulfan, melphalan) and nitrosureas (e.g. lomustine) demonstrate dose-dependent gonadotoxicity; the UK Children's Cancer and Leukaemia Group (CCLG) and British Fertility Society (BFS) have provided helpful "guesstimates" of the 'gonadotoxicity risk' of current common children's cancer treatment regimens (Table 1)^{4,7} in three broad categories – low (<20%), medium (20-80%) and high (>80%). Alkylating agents (e.g. cyclophosphamide) are most likely to cause future subfertility, whilst protocols for acute lymphoblastic leukaemia (ALL) (vincristine, methotrexate, cytarabine) are least likely to do so.

Radiotherapy

Radiotherapy may affect fertility at more than one level on the hypothalamopituitary-testicular axis (Figure 1). Firstly, the testis is vulnerable to increasing doses of irradiation which differentially affect the seminiferous epithelium and Leydig cells (Table 2)^{8,9}. In adults, fractionated doses as low as 0.1 Gy may cause temporary azoospermia whilst doses of 2-3 Gy are likely to prevent spermatid recovery long-term. Larger doses of 10-12 Gy as used in total body irradiation damage both Leydig and Sertoli cell function and, if given concurrently with chemotherapy, have additive effects. LH hypersecretion can partially compensate for mild Leydig cell dysfunction with near-normal testosterone values (subclinical *hypergonadotrophic hypogonadism*), but the long-term impact on bone strength and cardiac health is unknown.

Cranial irradiation, used in high dose (>40 Gy) and primarily for centrally positioned brain tumours, may reduce hypothalamopituitary function and result in *hypogonadotrophic hypogonadism*¹⁰. In the developing child this manifests as pubertal delay or arrest, although in the context of pre-existing pituitary disease or tumour these are the more likely causative factors. By contrast, early or precocious puberty is a typical manifestation of any cranial injury to the young brain (including disease, surgery or irradiation) but its evolution does not preclude later pubertal arrest and eventual gonadotrophin deficiency affecting both LH-testosterone and FSH-spermatogenic pathways and causing impotence and subfertility respectively. Only the former can be treated with testosterone replacement therapy.

Disease

Occasionally, the disease itself can cause testicular dysfunction. Hodgkin's disease has been associated with pre-treatment abnormalities in semen quality even in the absence of testicular infiltration⁵. The pathogenesis of this phenomenon is poorly understood but is thought to relate to disease-induced inflammatory processes.

Current fertility preservation strategies

In mature adults, pre-treatment sperm cryopreservation has been the most successful method of fertility preservation since the 1950s. Spermatozoa are remarkably resistant to storage and freeze-thawing processes, and healthy offspring without congenital anomalies have been reported from sperm stored for up to 28 years¹¹. Separately stored "straws" of spermatozoa can be thawed as required and

used in fertility treatments either directly by intrauterine insemination (IUI) or by intracytoplasmic sperm injection (ICSI), a technique available since 1992 which has markedly reduced the number of viable spermatozoa required to single numbers¹².

Requesting and obtaining masturbatory semen samples from older post-pubertal males is relatively straightforward. However, even ill adults may fail to produce a specimen in this way. For these patients and younger boys unable to produce a sample due to psychological or sexual immaturity, religious or cultural beliefs, alternatives such as rectal electrostimulation, penile vibratory stimulation, surgical extraction (TESE) under general anaesthesia, and even experimental techniques such as testicular tissue storage have been considered but not widely practised.

To facilitate service delivery, what remains unclear is exactly when during pubertal maturation boys are able to spontaneously ejaculate semen containing viable sperm. Spermaturia – the appearance of sperm in the urine by retrograde ejaculation – has been documented in healthy boys as young as 11.7 years of age and at a minimum testicular volume of 4.7 ml with little or no pubic hair development (Tanner stage P1, G2) and before peak height velocity¹³. The age range for initial spermaturia however is wide and up to 17.5 years. Although it has been used as an estimate of true spermarche there is no documentation of what triggers spermatogenesis, its correlation with pubertal staging and the ability to voluntarily donate semen, whilst spermatozoa obtained from urine are less viable and hence unsuitable for cryopreservation.

Our own pioneering service for adolescent boys at University College London Hospital since 1999

Over 10 years ago, with the support of colleagues in child psychiatry, reproductive health and haematology/ oncology contributing to the development of age-appropriate information leaflets, awareness campaigns and streamlined risk-assessments and referrals, we set up a pioneering endocrine/ fertility assessment and counselling service for adolescents. This was targeted at males with cancer aged 12 to 18 years referred to our tertiary centre for high dose therapies.

The repeated 3-4 yearly audit cycles of service in a total of 222 boys over that time have demonstrated a surprisingly consistent and unchanging counselling rate of some 70% but with an appropriately greater prioritisation of those at highest 'gonadotoxicity risk' over time. However, the relative paucity (30.0%) of documented pubertal clinical examinations despite good biochemical marker measurements (70.3%) and high (68.4%) patient acceptability of the counselling process persists to date. 34.2% of the total cohort (and 65.0% of those actually attempting storage) banked viable sperm produced by masturbation, the youngest boys being 12.6 years at Tanner stage 3+ and/ or with a testicular volume of ≥ 8 mls¹⁴. Importantly, it was hormone parameters of virilisation (testicular volume, plasma LH and testosterone concentrations), not age *per se*, which correlated with successful storage, whilst parameters of spermatogenesis (plasma FSH concentration) determined the normality of sperm concentrations. The role of age came into play only as a

surrogate marker of pubertal development and was not independently predictive of outcome.

Prepubertal boys

There are currently no proven fertility preservation techniques for prepubertal boys (testicular volume <4 mls). Cryopreservation of diploid spermatogonial cells obtained by testicular biopsy for later post-cure auto-transplantation or *in vitro* maturation to spermatozoa (as currently debated in young women) is still experimental without successful conceptions, even in animal models. Auto-transplantation carries a theoretical risk of reintroducing malignant cells, particularly in haematological malignancies where the testes are potential sites of metastases. Other prophylactic techniques such as testicular shielding during radiotherapy or the administration of GnRH analogues or testosterone to render cell division quiescent and less susceptible to cytotoxins have limited, if any, practical success.

Legal, ethical and practical considerations

The storage and use of haploid gametes and embryos is governed by the Human Fertilisation and Embryology (HFE) Act¹⁵ – this mandates personal, not proxy, informed consent in line with reproductive rights. Thus, unlike other paediatric procedures – for instance consenting for an appendicectomy – parents cannot give valid consent on a teenager’s behalf; any minor (under 18 or 16 years of age respectively in England and Scotland) must be judged intellectually (“Gillick”) competent to consent without coercion. Written consent to disclosure, HIV, hepatitis B and C testing and the use of stored samples after death or mental incapacitation is additionally required at this difficult time. Paradoxically, the legal loophole in which diploid pre-pubertal testicular tissue does not fall under HFE jurisdiction until such time as it becomes haploid leaves pre-pubertal boys potentially open to harm from experimentation – such as surgical removal of pre-meiotic spermatogonia – under the Common Law of parental consent even if the sole intent to preserve fertility appears well intended.

There are few, if any, adolescent-tailored sperm banking and counselling facilities in the UK, and there is debate as to how adult services might be modified to meet their specific needs (e.g. the environment, written information and pornographic material provided). The increased press focus on fertility preservation and the National Institute of for Health and Clinical Excellence’s (formerly NICE) recommendations suggests such a service might be offered more widely. However, whilst the young age of these increasing number of survivors would indicate a need for longer-term storage, historically, few stored samples have ever been used. This would suggest adult survivors either retain or do not ultimately want fertility, but also that patients at highest risk of subfertility are those most heavily treated and likely to die from aggressive disease or treatment-related complications.

An adolescent fertility counselling service (Table 4)

The patient perspective

Oncologists may perceive reproduction as too sensitive and inappropriate a topic to broach with adolescents already undergoing a complex counselling and consent

process for cancer treatment¹⁶. However, the few studies in this area indicate surprising awareness amongst adolescents, who in fact welcome discussion and choice – a positive experience at this stage of their disease¹⁷.

Pre-treatment fertility assessment

The young teenager has unique ethicolegal, physical, psychosexual and intellectual needs very different from the fully mature adult. To be fit for purpose any adolescent fertility service should routinely, consistently and reliably measure and record pubertal staging and testicular volumes; this is but one step further than the routine examination of the testes required to exclude malignant involvement. Paired with pre-treatment plasma endocrine biochemistry (LH, FSH, testosterone \pm inhibin B where possible), this baseline assessment should form the gold standard against which service adherence might be judged and audited. It is vital not only to inform the counselling process and the individual patient's chances of producing a viable sperm sample but also to assess future serial change indicative of gonadotoxicity and/ or spermatid recovery with time. Since we have found that immediately prior (within 6 days) commencement of chemotherapy is highly likely to reduce sample viability by $>75\%$ ¹⁴, if it is to be successful in those at high risk judged from Table 2, fertility preservation should be given earlier and higher priority in the cancer counselling process, even to the point of delaying cancer treatment to allow several attempts where possible.

Post-treatment fertility assessment

The long-term follow-up of teenage cancer survivors has not to date emphasised routine fertility assessment, semen analysis and/ or sperm banking (against a future relapse) in those still minors (<18 years) at the end of cancer therapy. However, counselling young boys and supporting them to donate interval post-treatment semen samples together with routine pubertal and biochemical assessment would provide the data needed to inform future age-appropriate services and sperm storage facilities based on the true gonadotoxicity (and time to recovery) of different cancer treatment regimens and their clinical correlates. This would concur with the 2003 BFS consensus recommendation that where sperm was not cryopreserved before treatment, a further opportunity at least 3 months from the end of treatment (to reduce the risk of DNA damage) should be offered⁷. For the counselling process to be truly complete, it should ensure understanding of the difference between potency (likely to be preserved or otherwise easily replaced) and fertility; for those unable or choosing not to cryopreserve sperm pre-treatment, there is still well-documented potential for recovery of natural fertility even 5 years after treatment¹⁸ and the consequent need for contraception in all. Testicular self-examination should be encouraged in the older teenager to monitor for tumour relapse or secondary malignancies.

Conclusions

As ART continues to rapidly evolve, fertility counselling in adolescence presents a specific and growing challenge. Government-level debate on the future role of the HFEA would do well to give consideration to the needs of this cohort who are increasingly campaigning for protection of their reproductive rights through patient

groups such as the Teenage Cancer Trust. Meanwhile, clinicians can be reassured that the large majority of young teenagers welcome the discussion and can exercise appropriate informed choice even in the context of a life-threatening illness. There should thus be ample opportunity to discuss options available to them both before and after cancer treatment with concurrently improved documentation of consent and clinical examination to make the service truly tailored to the adolescent.

Figures & Tables

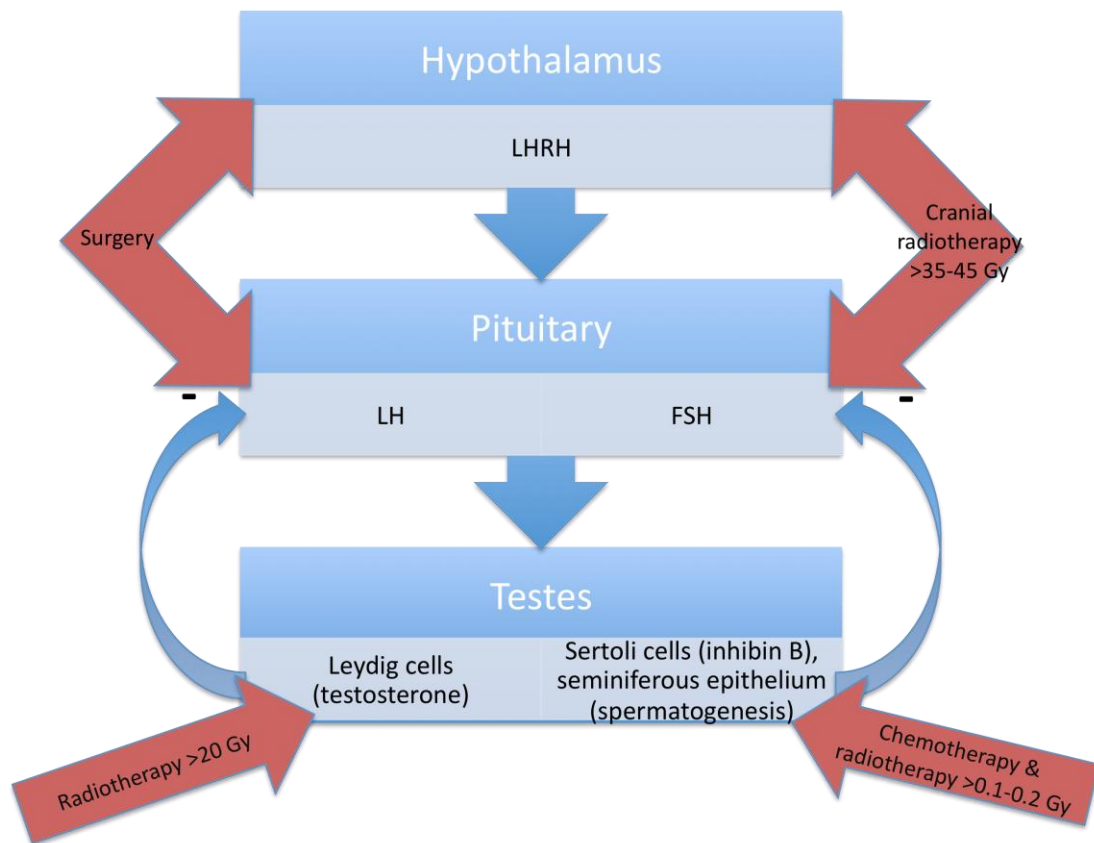


Figure 1: Schematic representation of the hypothalamic-pituitary-testicular axis and potential sites of disruption secondary to various cancer treatment modalities as indicated by the red arrows. Thin blue arrows indicate physiological negative feedback mechanisms. LH, luteinising hormone; FSH, follicular stimulating hormone; Gy, gray.

Low risk (<20%)	Medium risk (20-80%)	High risk (>80%)
Acute lymphoblastic leukaemia Wilms' tumour Soft tissue sarcoma: stage I Germ cell tumours: with gonadal preservation and no radiotherapy Retinoblastoma Brain tumour: surgery only or cranial irradiation <24 Gy	Acute myeloblastic leukaemia Hepatoblastoma Osteosarcoma Ewing's sarcoma Soft tissue sarcoma: stage II-III Neuroblastoma Non-Hodgkin lymphoma Hodgkin disease: alternating ('hybrid') therapy Brain tumour: craniospinal radiotherapy or cranial irradiation >24 Gy	Total body irradiation Chemotherapy conditioning for bone marrow transplant Localised radiotherapy: pelvic/testicular Hodgkin disease: alkylating agent-based therapy Soft tissue sarcoma: metastatic/stage IV Ewing's sarcoma: metastatic/stage IV

Table 1: Best estimate guidance for estimating risk of subfertility from various treatment modalities based on current regimens used in common childhood and adolescent cancers^{4,7}

Dose of irradiation	Effects on fertility
0.1-0.2 Gy	Transient impairment of spermatogenesis
>4 Gy	Risk of permanent impairment of spermatogenesis
20-30 Gy	Leydig cell dysfunction
35-45 Gy (cranial)	Hypogonadotropic hypogonadism

Table 2: Effects of increasing doses of irradiation on hypothalamopituitary-testicular dysfunction. Note that the impact of total body irradiation is difficult to quantify^{8,9}.

Key points in fertility counselling of adolescent boys with cancer
<p><u>Pre-treatment:</u></p> <p>Counsel within adolescent-tailored facilities (ideally)</p> <p>Counsel before commencement of treatment</p> <p>Allow sufficient opportunity to bank samples before treatment</p> <p>Clinical assessment – pubertal stage, testicular volume, best estimate of subfertility risk</p> <p>Biochemical assessment – LH, FSH, testosterone, inhibin B</p> <p>Gillick competence & informed consent including virology (HIV, hepatitis B & C)</p>
<p><u>Post-treatment:</u></p> <p>Streamlined late effects follow-up (may have other endocrine toxicities)</p> <p>Encourage post-treatment semen analysis (do not bank before 3 months)</p> <p>Contraceptive advice</p> <p>Testicular self-examination</p>

Table 3: Summary of main points in the assessment and counselling for fertility preservation in adolescent boys.

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